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(54) Title: ISOLATED FORMYLATED BACTERIAL PEPTIDES, NUCLEIC ACID MOLECULES AND USES THEREOF

(57) Abstract

The present invention provides product and process for protectingan animal from *Mycobacterium* infection. Such products includepeptides, and nucleic acid molecules encoding such peptides, thatare capable of eliciting an immune response sufficient to reduce the numbers of *Mycobacterium* in an infected animal. Methods to administer the products of the present invention are also provided. In particular, the present invention provides *Mycobacterium* peptides having amino terminal formylated methionines.

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ISOLATED FORMYLATED BACTERIAL PEPTIDES, NUCLEIC ACID MOLECULES AND USES THEREOF

This invention was made in part with government support under AI-35182, awarded by the National Institutes of Health. The government has certain rights to this invention.

FIELD OF THE INVENTION

The present invention relates to a product and process for protecting an animal from *Mycobacterium* infection and in particular, from *Mycobacterium tuberculosis*. The product of the present invention concerns an isolated antigenic peptide having an amino-terminal formylated methionine.

BACKGROUND OF THE INVENTION

Throughout history, tuberculosis has been one of the most deadly diseases. In 1991, it was estimated that about 1.7 billion people were infected with *Mycobacterium tuberculosis* (Snider et al., in *Tuberculosis*, ASM Press, Washington D.C. (Barry Bloom, ed.), 1994, p. 4). An estimated 2.7 million people died from tuberculosis in 1992 (Snider et al., *ibid.*). In recent years, the epidemic of the human immunodeficiency virus (HIV) has caused marked increases in the incidence of tuberculosis worldwide because HIV-infected individuals are immunocompromised and, therefore highly susceptible to secondary infections. It is estimated that greater than 5 million people have dual HIV and *Mycobacterium* infection, with increasing numbers occurring in industrialized countries.

Traditional therapy for tuberculosis is treatment of patients with antibiotics. Over the past 30 years, however, efforts to combat infection with antibiotics has been thwarted by the emergence of multi-drug resistant strains of *Mycobacterium tuberculosis*. With the emergence of strains resistant to existing drugs, the attention of the medical community has turned to the development of alternative anti-*Mycobacterium* agents, such as vaccines. Different forms of vaccines have been considered, including non-pathogenic forms of whole bacteria, whole proteins and peptides of proteins (see, for example, Young et al., *Bull. Int. Union Tuberc. Lung Dis.* 66:47-51, 1991; Wiker et al., *Microbiol. Rev.* 56:648-661, 1992; Borremans et al., *Infection and Immunity* 57(10):3123-3130, 1989; Content et al., PCT Publication No. WO 91/04272, published April 4, 1991; and Content et al., PCT Publication No. WO 92/14823, published September 3, 1993). The effectiveness of such vaccines to protect an animal from *Mycobacterium* infection, has been inefficient and unpredictable. In addition, because certain vaccines comprise whole bacteria, administration of vaccines creates false positives which interfere with purified protein derivative (PPD) tuberculosis screening tests using *Mycobacterium* proteins.

Thus, there remains a need to develop therapeutic reagents that are capable of inducing a protective immune response in an animal against *Mycobacterium*, thereby prev nting the occurrence of tuberculosis and the spread of the pathogen.

SUMMARY OF THE INVENTION

The present invention provides an antigenic peptide having an amino-terminal formylated methionine that is protective against *Mycobacterium*, as well as the use of the peptide to protect an animal from *Mycobacterium* infection, thereby alleviating the occurrence of disease and the spread of the infectious pathogen. The invention is particularly advantageous in that it provides an antigenic peptide that is capable of binding to a MHC molecule in such a manner that the immune system of an animal is stimulated in such a manner that infection of an animal by *Mycobacterium* can be prevented or reduced. Another advantageous aspect of a peptide of the present invention is that, unlike the most frequently used *Mycobacterium* vaccine (BCG) which is a whole cell vaccine, the present peptide does not interfere with commonly used *Mycobacterium* diagnostic skin tests such as PPD tests. In addition, unlike a whole cell vaccine such as BCG, use of a peptide of the present invention is not accompanied by the risk of reversion to virulence.

One embodiment of the present invention includes an isolated antigenic peptide having an amino-terminal formylated methionine, the peptide being capable of protecting an animal against *Mycobacterium* infection. In particular, a peptide of the present invention is derived from a *Mycobacterium* protein. A preferred antigenic peptide includes SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID

NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:16.

Another embodiment of the present invention includes an isolated nucleic acid molecule having a sequence encoding a peptide having an amino-terminal formylated methionine when the nucleic acid molecule is expressed in a bacteria cell, the peptide being capable of protecting an animal from *Mycobacterium* infection. A preferred nucleic acid molecule of the present invention includes SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15. The present invention also includes recombinant molecules and recombinant cells that include antigenic peptide nucleic acid molecules of the present invention.

Yet another embodiment of the present invention is a therapeutic composition to protect an animal from infection by an intracellular pathogen, the composition comprising: (1) an isolated antigenic peptide having an amino-terminal formylated methionine; and (2) a pharmaceutically acceptable carrier, in which the antigenic peptide is capable of protecting an animal from *Mycobacterium* infection. A preferred therapeutic composition comprises at least one antigenic peptide including SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:16. Another preferred therapeutic composition comprises a nucleic acid sequence encoding an antigenic peptide capable of protecting an animal from *Mycobacterium* infection when the nucleic acid

sequence is transformed into a bacterial cell, the nucleic acid sequence being operatively linked to one or more transcription control sequences. Also included is a method to protect an animal from *Mycobacterium* infection by administering to the animal a therapeutic composition of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 illustrates flow cytometry staining profiles of H-2M3 molecule expression on Mtb peptide treated cells.

Fig. 2 illustrates the binding of ^{F}Met Mtb peptides to H-2M3 molecules.

Fig. 3 illustrates the lack of binding of non-formylated Mtb peptides to H-2M3 molecules.

Fig. 4 illustrates the ability of ^{F}Met Mtb peptides to elicit cytotoxic T cell activity against Mtb-H37Rv-infected macrophage cells.

Fig. 5 illustrates cytotoxic T cell activity against C57B16 target cells in the absence of ^{F}Met-Peptides.

Fig. 6 illustrates cytotoxic T cell activity against C57B16 target cells in the presence of ^{F}Met-Peptide B.

Fig. 7 illustrates cytotoxic T cell activity against C57B16 target cells in the presence of ^{F}Met-Peptide C.

Fig. 8 illustrates cytotoxic T cell activity against C57B16 target cells in the presence of ^{F}Met-Peptide E.

Fig. 9 illustrates cytotoxic T cell activity against B10.BR target cells in the absence of ^{F}Met-Peptide.

Fig. 10 illustrates cytotoxic T cell activity against B10.BR target cells in the presence of ^{F}Met-Peptide B.

Fig. 11 illustrates cytotoxic T cell activity against B10.BR target cells in the presence of ^{F}Met-Peptide C.

Fig. 12 illustrates cytotoxic T cell activity against B10.BR target cells in the presence of ^{F}Met-Peptide E.

Fig. 13 illustrates the effect of ^{F}Met-Peptide immunization of mice on Mtb dissemination to the spleen.

Fig. 14 illustrates the effect of ^{F}Met-Peptide immunization of mice on Mtb dissemination to the lung.

Fig. 15 illustrates that ^{F}Met-Peptide E is capable of eliciting cytotoxic T cell activity in human PBMCs.

Fig. 16 illustrates that ^{F}Met-Peptide B is capable of eliciting cytotoxic T cell activity in human PBMCs.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a product and process for protecting an animal from *Mycobacterium* infection. In particular, the product includes an antigenic peptide having an amino-terminal formylated methionine (also referred to herein as N-formylated or ^{F}Met). The process includes administering the peptide to an animal to protect the animal from *Mycobacterium* infection.

The ability of an antigenic peptide to protect an animal from infection by an intracellular pathogen is dependent upon the complex biology involved in the use of peptides to control T cell activity in an animal. Typically, an intracellular pathogen, such as

Mycobacterium, invades a cell of an animal (i.e., a host cell) and propagates within such cell. A protein of the intracellular pathogen can undergo proteolysis in the host cell resulting in the formation of various peptides. In order for a peptide formed by intracellular proteolytic cleavage to elicit a protective immune response mediated through the major histocompatibility complex (MHC) class I pathway, the peptide must be: (1) capable of binding to an MHC class I molecule; (2) transported to a location in the host cell (e.g., the endoplasmic reticulum (ER) so that th peptide can bind to an MHC class I molecule to form an MHC:Peptide complex; (3) transported in sufficiently abundant amounts so that the number of MHC:Peptide complexes needed to elicit an immune response are created; and (4) after being complexed to an MHC molecule, transported to the plasma membrane of the host cell so that the complex is available to bind to a T cell receptor (TCR) on the surface of a T lymphocyte (T cell). Engagement of a TCR by an MHC:Peptide complex can elicit an immune response by controlling the activity of the T cell bearing the TCR.

Non-naturally occurring peptides can be produced that essentially mimic the protective activity of a naturally-occurring peptide formed by intracellular proteolytic cleavage. To be protective, non-naturally occurring peptides must be able to bind to MHC molecules in sufficient amounts to elicit an immune response, and be presented on the surface of a cell so that the MHC:Peptide complex is available to bind to TCR's. According to the

present invention, a peptide that is capable of eliciting a protective immune response based on the mechanism described immediately above is referred to as an antigenic peptide. An antigenic peptide of the present invention includes an isolated peptide that represents a naturally-occurring peptide (i.e., a protein fragment resulting from proteolytic cleavage of a *Mycobacterium* protein in a host cell) or a mimetope (described in detail below) of a naturally-occurring peptide that is capable of eliciting a protective immune response based on the mechanism described immediately above.

An antigenic peptide of the present invention is capable of binding to an MHC molecule. Examples of methods to test the binding ability of a peptide to an MHC molecule are disclosed in the Examples section. It is within the skill of one in the art to determine appropriate MHC molecules to use in a binding study based on the peptide being tested. It is believed that the binding of a peptide to an MHC molecule elicits an immune response by creating an epitope recognized by a T cell receptor (TCR), which is then bound by a TCR, resulting in the modification of the activity of the T cell bearing the TCR. In addition, upon administration to an animal, antigenic peptides of the present invention are capable of binding to a sufficient number of MHC molecules to control the activity of T cells (as described in more detail below), thereby eliciting a protective immune response against *Mycobacterium*.

According to the present invention, a peptide of the present invention is an isolated peptide. An isolated peptide refers to a peptide that is not in its natural milieu. As such, "isolated" does not necessarily reflect the extent to which the peptide has been purified. For example, a peptide that is considered isolated can be, without limitation: peptide alone, either synthesized by a method described below or removed from an animal; peptide bound to an MHC molecule, either synthesized by a method described below or removed from an animal; or peptide bound to an MHC molecule, which itself is bound to a lipid bilayer (e.g., a cell), either synthesized by a method described below or removed from an animal. An isolated peptide of the present invention can be obtained from its natural source, produced by proteolysis of a full-length protein or larger protein fragment, produced using recombinant DNA technology or synthesized using standard chemical peptide synthesis methods (for example, as described in the Example section below).

The term "infection" refers to the introduction and propagation (i.e., increase in numbers) of an intracellular pathogen in an animal. In the case of an intracellular pathogen, infectivity can be reliant upon the ability of the pathogen to invade a host cell or tissue and to multiply within such cell or tissue, as well as the efficiency with which the pathogen multiplies within and escapes from a host cell or tissue. According to the present invention, an antigenic peptide of the present

invention is also referred to as a "protective peptide." A protective peptide refers to a peptide that is capable of eliciting a protective immune response. A protectiv immune response can result in a decrease and/or prevent the increase in the number of pathogenic microorganisms in the tissue of an animal. Thus, administration of a protective peptide to an animal can ameliorate and/or prevent disease by reducing and/or preventing the increase of the number of pathogenic microorganisms in the tissue of an animal compared with untreated animals. The effectiveness of a peptide to affect the number of pathogenic microorganisms in the tissue of an animal can be determined using methods standard in the art. For example, as described in detail in the Examples section, peptides can be administered to mice contacted with pathogenic *Mycobacterium* and colony counts for bacterial pathogens performed on biopsies of infected tissue removed from the mice.

An infected animal is referred to as a "host animal" of an infectious pathogen. In addition, a cell infected by an intracellular pathogen in referred to as a "host cell." It is also to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a compound refers to one or more compounds. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein.

A suitable antigenic peptide of the present invention is capable of altering the activity of a T cell upon administration of the peptide to an antigen presenting

cell. In particular, antigenic peptide of the present invention is capable of altering the activity of a T cell upon administration of the peptide to an animal, thereby protecting the animal from *Mycobacterium* infection. Preferably, an antigenic peptide of the present invention is capable of binding to an MHC molecule that itself is capable of binding to a naturally-occurring *Mycobacterium* peptide (i.e., a *Mycobacterium*-specific MHC molecule). A naturally-occurring *Mycobacterium* peptide refers to a peptide formed by proteolytic cleavage of a *Mycobacterium* protein on a *Mycobacterium* that has infected a host cell. In particular, an antigenic peptide of the present invention is capable of binding to an MHC class I molecule in such a manner that an epitope is formed that can be recognized by a TCR on a $CD8^+$ T cell, thereby causing the stimulation of a cytotoxic T cell (T_c cell). TCR recognition refers to the ability of a TCR to bind to an MHC:peptide complex in such a manner that the activity of the T cell bearing the TCR is modified. For example, an antigenic peptide of the present invention is capable of forming an MHC:Peptide complex that results in substantial chromium release from target cells in cytotoxic T lymphocyte (CTL) assays according to the methods described in detail in the Examples section.

Suitable MHC class I molecules to which an antigenic peptide of the present invention can bind include non-polymorphic MHC class I molecules. Without being bound by theory, an isolated N-formylated peptide of the present

invention is capable of binding to a non-polymorphic MHC class I molecule. A non-polymorphic MHC molecule comprises an MHC molecule that is capable of binding to an N-formylated peptide and that displays less variation between alleles than classical MHC class I molecules that show substantial allelic variation. The ability of a peptide to bind to non-polymorphic MHC molecules makes the peptide suitable for administration to populations of patients rather than to individual patients.

An isolated peptide of the present invention can bind to an MHC molecule with sufficient efficiency such that a suitable number of MHC:Peptide complexes are formed on a cell to alter the activity of a T cell upon TCR engagement of the MHC:Peptide complexes. For example, it is known in the art that multiple TCR's must bind to MHC:Peptide complexes to alter the activity of a T cell bearing the TCR's. Thus, a single MHC:Peptide complex cannot alter the activity of a T cell because only a single TCR on the T cell will be engaged. Preferably, the binding efficiency of isolated peptides of the present invention to MHC molecules is sufficient to produce from about 3 to about 300 MHC:Peptide complexes, more preferably from about 5 to about 250 MHC:Peptide complexes, and even more preferably from about 10 to about 200 MHC:Peptide complexes per cell upon administration of about 1 μg to about 100 μg of peptide to an animal.

When administered to an animal, an antigenic peptide of the present invention is capable of lowering the number

of *Mycobacterium* in an infected organ, when compared with an infected organ of an untreated animal, by at least about 10 percent, more preferably by at least about 25 percent, and even more preferably by at least about 50 percent. Procedures to determine the reduction in *Mycobacterium* numbers are described in detail below in the Examples section.

An antigenic peptide suitable for protecting an animal from *Mycobacterium* infection includes an antigenic peptide having an amino-terminal formylated methionine, in which the peptide is derived from (i.e., having the same amino acid sequence as) a *Mycobacterium* protein. An antigenic peptide of the present invention is not derived from a mitochondrion. A preferred antigenic peptide is derived from *Mycobacterium tuberculosis* (referred to herein as *M. Tuberculosis* or Mtb), *Mycobacterium leprae*, *Mycobacterium avium* and/or *Mycobacterium bovis*. A more preferred antigenic peptide is derived from *M. Tuberculosis*. An even more preferred antigenic peptide is derived from a protein including an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and/or *M. Tuberculosis* GenBank Accession No. M76712.

The length of an antigenic peptide of the present invention is any suitable length that enables the peptide to bind to an antigenic peptide groove of an MHC molecule. A preferred length of an antigenic peptide of the present

invention is from about 5 amino acids to about 20 amino acids, more preferably from about 7 amino acids to about 15 amino acids, and even more preferably from about 8 amino acids to about 11 amino acids.

An antigenic peptide of the present invention also includes mimetopes of such peptides. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the protective characteristics of an antigenic peptide of the present invention. A mimetope can be a peptide, the amino acid sequence of which has been modified by, for example, adding, deleting and/or substituting one or more amino acid residues but that still retains protective characteristics against *Mycobacterium* infection. Other examples of mimetopes include, but are not limited to, protein-based compounds, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof having desired protective activity, that have been identified using information obtained from, for example, the structure and/or binding characteristics of an isolated peptide of the present invention. A mimetope can be obtained, for example, from libraries of natural or synthetic compounds, in particular from chemical or combinatorial libraries (i.e., libraries of compounds that differ in sequence or size but that have the same building blocks and are capable of protecting an animal against *Mycobacterium* infection, as

disclosed herein; see for example, U.S. Patent Nos. 5,010,175 and 5,266,684 of Rutter and Santi) or by rational drug design. In a rational drug design procedure, the three-dimensional structure of a peptide of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or X-ray crystallography. The two- and/or three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modelling. It may also be necessary to determine the three-dimensional conformation of a peptide when it is bound to an MHC molecule. The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source (e.g., plants, animals, bacteria and fungi).

It is predictable to one of skill in the art that certain additions, deletions and/or substitutions can be made to a peptide of the present invention that will not effect the peptide's ability to bind to an MHC molecule and protect against *Mycobacterium* infection. The functional impact of an alteration to a peptide of the present invention can easily be tested using, for example, a protection assay as described in detail below in the Examples section. Preferably, an antigenic peptide mimetope that comprises a peptide is at least about 50% identical, more preferably at least about 75% and even more preferably at least about 85% identical to the amino acid sequence of an antigenic peptide of the present invention.

A preferred antigenic peptide of the present invention is a peptide encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a nucleic acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and/or *M. Tuberculosis* GenBank Accession No. M76712. A more preferred antigenic peptide of the present invention is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15 (as disclosed herein).

As used herein, stringent hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules, including oligonucleotides, are used to identify molecules having similar nucleic acid sequences. Such standard conditions are disclosed, for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press.

Particularly preferred isolated peptides of the present invention include of ^{F}Met-Ala-Asn-Pro-Phe-Val-Lys-Ala-Trp-Lys-Tyr (SEQ ID NO:2, wherein the amino terminal Met is formylated), ^{F}Met-Gln-Leu-Val-Asp-Arg-Val-Arg-Gly-Ala-Val (SEQ ID NO:4, wherein the amino terminal Met is formylated), ^{F}Met-Thr-Phe-Phe-Glu-Gln-Val-Arg-Arg-Leu-Arg (SEQ ID NO:6, wherein the amino terminal Met is formylated), ^{F}Met-Ala-Thr-Thr-Leu-Pro-Val-Gln-Arg-His-Pro

(SEQ ID NO:14, wherein the amino terminal Met is formylated), and truncated forms thereof, wherein standard three letter amino acid codes are used and wherein ^{F}Met symbolizes a formylated amino terminal methionine. Preferred truncated forms of isolated peptides of the present invention include ^{F}Met-Ala-Asn-Pro-Phe-Val-Lys-Ala (SEQ ID NO:8, wherein the amino terminal Met is formylated), ^{F}Met-Gln-Leu-Val-Asp-Arg-Val-Arg (SEQ ID NO:10, wherein the amino terminal Met is formylated), ^{F}Met-Thr-Phe-Phe-Glu-Gln-Val-Arg (SEQ ID NO:12, wherein the amino terminal Met is formylated) and ^{F}Met-Ala-Thr-Thr-Leu-Pro-Val-Gln (SEQ ID NO:16, wherein the amino terminal Met is formylated).

Another embodiment of the present invention includes a formula of isolated peptides of the present invention. A suitable formula of the present invention includes any combination of two or more isolated peptides of the present invention. It is within the skill in the art to choose effective combinations of peptides to create a formulation of the present invention, dependent upon for example, the patient being treated and whether the peptide is being administered as a preventative (i.e., vaccine) or ameliorating reagent. A preferred formula of the present invention contains any combination of two or more peptides including peptides represented by SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and/or SEQ ID NO:16. A more preferred formula of

the present invention contains a combination of peptides represented by SEQ ID NO:2 and SEQ ID NO:8.

Another embodiment of the present invention is an isolated nucleic acid molecule that, when expressed in a bacteria cell, encodes a peptide having an amino-terminal formylated methionine, the peptide being capable of protecting an animal from *Mycobacterium* infection. A nucleic acid molecule encoding an antigenic peptide of the present invention can include partial coding regions of an isolated natural *Mycobacterium* protein encoding gene or a homologue thereof. A nucleic acid molecule of the present invention can also include one or more regulatory regions.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that is not in its natural milieu (i.e., that has been subject to human manipulation). As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated nucleic acid molecule can include DNA, RNA, or derivatives of either DNA or RNA.

An isolated nucleic acid molecule of the present invention can be obtained from its natural source either as an entire (i.e., complete) gene or a portion thereof capable of encoding an antigenic peptide of the present invention. An isolated nucleic acid molecule encoding an antigenic peptide of the present invention (also referred to herein as an antigenic peptide-encoding nucleic acid molecule) can also be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR)

amplification, cloning) or chemical synthesis. Isolated antigenic peptide-encoding nucleic acid molecules include natural nucleic acid molecules and homologues thereof, including, but not limited to, natural allelic variants and modified nucleic acid molecules in which nucleotides have been inserted, deleted, substituted, and/or inverted in such a manner that such modifications do not substantially interfere with the nucleic acid molecule's ability to encode an antigenic peptide of the present invention.

A homologue of an antigenic peptide-encoding nucleic acid molecule of the present invention can be produced using a number of methods known to those skilled in the art (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989). For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. Homologues of a nucleic acid molecule of the present invention can be selected from a mixture of modified nucleic acids by screening for the function of the

peptide encoded by the nucleic acid molecule (e.g., the ability of the peptide to bind to an MHC molecule and/or elicit an immune response against *Mycobacterium* infection).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes at least one of the antigenic peptides of the present invention, examples of such peptides being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a protective peptide.

A suitable nucleic acid molecule of the present invention comprises a portion of a *Mycobacterium* gene. A preferred nucleic acid molecule comprises a portion of a *Mycobacterium* gene including *M. Tuberculosis* Genbank Accession No. M69187, *M. Tuberculosis* Genbank Accession No. M27016 X53898, *M. Tuberculosis* Genbank Accession No. X57229 and/or *M. Tuberculosis* Genbank Accession No. M76712. A more preferred nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid molecule including 5′ ATG GCC AAT CCG TTC GTT AAA GCC TGG AAG TAC 3′ (SEQ ID NO:1), 5′ ATG CAG CTT GTT GAC AGG GTT CGT GGC GCC GTC (SEQ ID NO:3), 5′ ATG ACG TTC TTC GAA CAG GTG CGA AGG TTG CGG 3′ (SEQ ID NO:5), 5′ ATG GCC ACC ACC CTT CCC GTT CAG CGC CAC CCG 3′ (SEQ ID NO:13), and truncated forms

thereof. Preferred truncated forms include 5′ ATG GCC AAT CCG TTC GTT AAA GCC 3′ (SEQ ID NO:7), 5′ ATG CAG CTT GTT GAC AGG GTT CGT 3′ (SEQ ID NO:9), 5′ ATG ACG TTC TTC GAA CAG GTG CGA 3′ (SEQ ID NO:11) or 5′ ATG GCC ACC ACC CTT CCC GTT CAG 3′ (SEQ ID NO:15). A more preferred nucleic acid molecule includes a nucleic acid sequence represented by SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:15.

The present invention includes a nucleic acid molecule of the present invention operatively linked to one or more transcription control sequences to form a recombinant molecule. The phrase "operatively linked" refers to linking a nucleic acid molecule to a transcription control sequence in a manner such that the molecule is able to be expressed when transformed into a host bacterial cell. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in a bacterial cell. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in attenuated bacteria pathogens. More preferred transcription control sequences include those which function in attenuated bacteria of the genera

Mycobacterium, *Listeria*, *Escherichia*, *Bacillus*, *Pseudomonas* and *Salmonella*. Even more preferred transcription control sequences include those which function in Bacille Calmette-Guerin (BCG), *Salmonella typhimurium* UK-1 $_{x}$3987 and *Salmonella typhimurium* SR-11 $_{x}$4072. Examples of suitable transcription control sequences of the present invention include, but are not limited to, transcription control sequences that regulate the expression of proteins including internalin, listeriolysin and *Mycobacterium* heat shock protein. Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with a *Mycobacterium* gene represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and/or *M. Tuberculosis* GenBank Accession No. M76712. Recombinant molecules of the present invention, which can be either DNA or RNA, can also contain additional regulatory sequences, such as translation regulatory sequences, origins of replication, and other regulatory sequences that are compatible with a bacterial cell.

Preferred recombinant molecules of the present invention include a recombinant molecule containing one or more nucleic acid molecules described in detail herein. A recombinant molecule containing two or more nucleic acid molecules can be designed such that each nucleic acid molecule can be expressed using the same or different regulatory control sequences.

It may be appreciated by one skilled in the art that use of recombinant DNA technologies can improve expression of transfected nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a bacteria cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals (e.g., promoters, operators, enhancers), substitutions or modifications of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences), modification of nucleic acid molecules of the present invention to correspond to the codon usage of the bacteria cell, and deletion of sequences that destabilize transcripts.

One embodiment of the present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of protecting that animal from a *Mycobacterium* infection. Therapeutic compositions of the present invention include at least one of the following protective compounds: (a) an antigenic

peptide of the present invention, or a mimetope thereof, and (b) an isolated nucleic acid molecule encoding an antigenic peptide of the present invention. Preferred *Mycobacterium* to target are heretofore disclosed. Examples of antigenic peptides and nucleic acid molecules encoding antigenic peptides, are disclosed above.

A therapeutic composition of the present invention can be administered to an animal as a vaccine to prevent *Mycobacterium* infection in an animal or as a medicinal reagent to treat an existing *Mycobacterium* infection in an animal. Vaccines comprising antigenic peptides of the present invention are referred to herein as peptide-based vaccines. Vaccines comprising nucleic acid molecules of the present invention are referred to herein as recombinant cell vaccines.

Therapeutic compositions of the present invention can be formulated in a pharmaceutically acceptable carrier that the animal to be treated can tolerate. Examples of such carriers include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate

buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, m- or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the carrier can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

Pharmaceutically acceptable carriers of the present invention can further comprise immunopotentiators, such as adjuvants or delivery vehicles. Adjuvants are typically substances that generally enhance the immune response of an animal to a specific antigen. Suitable adjuvants include those adjuvants that can be administered to humans. Preferred adjuvants for use with a therapeutic composition of the present invention include, but are not limited to, aluminum-based salts; calcium-based salts; silica; gamma interferon; interleukin-12 (IL-12) and other commercially available adjuvants. A preferred adjuvant for use with a recombinant-based vaccine of the present invention includes, but is not limited to, a nucleic acid molecule encoding a cytokine protein. Preferably, a cytokine-encoding nucleic acid molecule encodes a cytokine including granulocyte macrophage colony stimulating factor (GM-CSF),IL-12, tumor necrosis factor α (TNF-α), macrophage colony stimulating factor (M-CSF), interleukin-1 (IL-1)

and/or interleukin-6 (IL-6), with GM-CSF and IL-12 being more preferred.

Suitable delivery vehicles of the present invention include compounds that are capable of delivering (i.e., carrying) a therapeutic composition of the present invention. Preferred vehicles to deliver a peptide-based vaccine of the present invention include compounds that are capable of increasing the half-life of the vaccine in a treated animal. Such delivery vehicles include, for example, polymeric controlled release formulations (e.g., biocompatible polymers, biodegradable polymeric implants, capsules, microcapsules and microparticles), artificial and natural lipid-containing compositions (e.g., liposomes, lipospheres, micelles and cells), transdermal delivery systems, oils, esters, and glycols. More preferred vehicles to deliver a peptide-based vaccine include, but are not limited to, liposomes. Suitable liposomes for use with the present invention include any liposome. Preferred liposomes of the present invention include those liposomes standardly used in, for example, peptide delivery methods known to those of skill in the art.

A recombinant cell vaccine of the present invention comprises at least one nucleic acid molecule encoding an antigenic peptide, transformed into a delivery vehicle comprising a bacteria cell, to form a recombinant cell. Preferred bacteria cells for use as delivery vehicles include, but are not limited to, any attenuated bacteria cell. More preferred bacteria cells for use as delivery

vehicles include attenuated bacteria of the genera *Mycobacterium*, *Escherichia*, *Bacillus*, *Pseudomonas*, *Salmonella* or *Listeria* cells, with *Mycobacterium*, *Salmonella* or *Listeria* cells being even more preferred. Preferred *Mycobacterium* cells for use as delivery vehicles include BCG. Preferred *Salmonella* cells for use as delivery vehicles include *Salmonella typhimurium* UK-1 $_{x}$3987 and Salmonella *typhimurium* SR-11 $_{x}$4072.

Recombinant cell vaccines can be used to introduce protective peptides of the present invention into the immune systems of animals. For example, recombinant molecules comprising antigenic peptide nucleic acid molecules of the present invention operatively linked to one or more transcription control sequences that function in an attenuated bacteria cell can used to transform attenuated bacteria cells. The resultant recombinant bacterial cells are then introduced into the animal to be protected. Preferred attenuated bacteria cells are those which are facultative intracellular parasites. Thus, the live recombinant cell vaccine strains can persist for long periods in a cell of a patient, producing therapeutic peptides of the present invention. Production of the peptide inside the patient's cell has the added advantage of making the peptide accessible to the MHC molecule pathway of the patient's cell. In this manner, patients treated with a recombinant cell vaccine can develop an immune response to the peptide. .

A delivery vehicle of the present invention can be modified to target to a particular site in an animal. A "target site" refers to a site, preferably a particular cell, in an animal to which one desires to deliver a therapeutic composition. For example, a target site can be an antigen presenting cell and/or an organ typically infected by *Mycobacterium*, such as a liver, a spleen and/or a lung. Preferred antigen presenting cells to target include dendritic cells, macrophages and B lymphocytes. Suitable modifications include manipulating the chemical formula of a delivery vehicle and/or introducing into the vehicle a compound capable of specifically targeting the vehicle to a preferred site, for example, a preferred antigen presenting cell. Specifically targeting refers to causing a delivery vehicle to bind to a particular cell by the interaction of the compound in the vehicle to a molecule on the surface of the cell. Suitable targeting compounds include ligands capable of selectively (i.e., specifically) binding another molecule at a particular site. Examples of such ligands include antibodies, antigens, receptors and receptor ligands. For example, an antibody specific for an antigen found on the surface of a macrophage cell can be introduced to the outer surface of a delivery vehicle so as to target the delivery vehicle to the antigen presenting cell. Manipulating the chemical formula of a delivery vehicle includes for example, adding a chemical to a lipid formula of a liposome that alters the charge of the lipid bilayer of the liposome so that the

liposome fuses with particular cells having particular charge characteristics.

In order to protect an animal from *Mycobacterium* infection, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting that animal from the infection. For example, an isolated peptide or mimetope thereof, when administered to an animal in an effective manner, is able to elicit an immune response, preferably including both a humoral and cellular response but in particular a cellular response, that is sufficient to protect the animal from *Mycobacterium* infection. Nucleic acid molecules of the present invention can also be administered in an effective manner, thereby reducing *Mycobacterium* infection.

Therapeutic compositions of the present invention can be administered to any animal infected with *Mycobacterium*, preferably to mammals, and more preferably to humans and cattle. Particularly preferred animals to protect include humans.

Acceptable protocols to administer therapeutic compositions of the present invention in an effective manner include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. For example, it will be obvious to one of skill in the art that the size and number of doses administered to an animal is dependent

upon the existence or extent of an infection and the response of an individual patient to a treatment. For example, a more extensive infection may require larger and/or more doses than a less extensive infection. In some cases, however, a patient having a more extensive infection may require smaller and/or fewer doses than a patient with a less extensive infection, if the patient with the more extensive infection responds more favorably to the therapeutic composition than the patient with the less extensive infection. In addition, administration of a therapeutic composition as a vaccine may require fewer and/or smaller doses than administration of a therapeutic composition as a medicinal reagent. Thus, it is within the scope of the present invention that suitable sizes and number of doses includes any amounts required to prevent infection or cause regression of an infection.

A suitable single dose is a dose that is capable of protecting an animal from *Mycobacterium* infection when administered one or more times over a suitable time period. For example, a preferred single dose of a peptide-based vaccine of the present invention is from about 10 microgram (μg) to about 10 milligrams (mg) of peptide per animal, more preferably from about 5 μg to about 5 mg of peptide per animal, and even more preferably from about 1 μg to about 1 mg of peptide per animal. Preferably, a recombinant cell vaccine is administered at doses ranging from about 10 to about 10^8 bacteria per animal. Similar amounts can be used for administration of peptide- and

recombinant cell-based medicinal reagents of the present invention.

Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster vaccinations preferably are administered when the immune response of the animal becomes unable to protect the animal from *Mycobacterium* infection. A preferred administration schedule of a peptide-based therapeutic composition is one in which from about 1 μg to about 1 mg of peptide per animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months.

Modes of administration of a peptide-based therapeutic composition can include a parenteral route, such as subcutaneous, intradermal, aerosol or intramuscular routes. A particularly preferred route by which to administer a peptide-based therapeutic composition of the present invention includes by an intramuscular, intradermal, intranasal and/or a subcutaneous route. A recombinant cell-based therapeutic composition of the present invention can be administered in a variety of ways but has the advantage that it can be administered orally. For example, both *Listeria* and *Salmonella* strains normally enter a host orally. Once in the intestine, they interact with the mucosal surface, normally to establish an invasive infection (as discussed in detail above). In addition, a recombinant cell-based therapeutic composition of the present invention can be administered by aerosol. For

example, *Mycobacterium* strains normally enter a host through the lung. Once in the lung, the bacteria can establish an invasive infection and produce therapeutic peptide in the lung.

It is within the scope of the present invention that a medicinal reagent of the present invention can be administered with additional protective compounds. In particular, a medicinal reagent of the present invention can be co-administered with suitable anti-*Mycobacterium* antibiotics, such as isoniazid, rifampin, ethambutol, cyprofoloxiacin, amikacin and pyraziamide.

One preferred embodiment of the present invention is the use of antigenic peptides and nucleic acid molecules encoding such peptides to protect an animal from tuberculosis. An antigenic peptide is preferably used to prevent propagation of *Mycobacterium* cells or destroy infected host cells by stimulating T_C cells to kill host cells of a patient that are infected with *Mycobacterium* and/or to prevent or reduce *Mycobacterium* infection by stimulating the production of cytokines that are effective in preventing or reducing *Mycobacterium* infection. Preferably, administration of an antigenic peptide of the present invention is effective to stimulate the release of interferon gamma, IL-12, TNF-α and/or IL-2.

Another embodiment of the present invention includes a therapeutic composition comprising an antigenic peptide of the present invention complexed with an MHC molecule. Peptide:MHC complexes for use as a therapeutic composition

can be prepared using a variety of methods. For example, an antigenic peptide can be mixed with MHC molecules to enable spontaneous association of the peptide to the MHC molecule. The association of the peptide to the MHC molecule can be stabilized using protein cross-linking reagents. Alternatively, a recombinant molecule can be created that comprises a nucleic acid sequence that encodes an antigenic peptide, in which that sequence is operatively linked to a nucleic acid sequence that encodes an MHC molecule. Certain methods to produce Peptide:MHC complexes *in vitro* are disclosed in U.S. Patent No. 5,260,422, issued November 9, 1993, by Clark et al.; U.S. Patent No. 5,194,425, issued March 16, 1993, by Sharma et al.; and U.S. Patent No. 5,130,297, issued July 14, 1992, by Sharma et al., which are incorporated herein by this reference in their entirety. Peptide:MHC complexes can also be isolated from cells removed from an animal infected with *Mycobacterium*. Preferred MHC molecules to use in a complex include human homologues of H-2M3.

According to the present invention, a therapeutic Peptide:MHC complex is attached to a lipid carrier that contains at least one protein that is capable of mediating signal transduction in a T cell that results in T cell activation. For example, a lipid carrier can contain the protein B7 which is capable of binding to CD28 on the surface of a T cell. The conjugation of B7 and CD28 assist the activation of a T cell in conjunction with the binding of the Peptide:MHC complex to a TCR. Preferred lipid

carriers include those disclosed herein, including mammalian cells, such as red blood cells, fibroblast cells, pluripotent progenitor cells, epithelial cells and neural cells.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example describes the ability of *Mycobacterium tuberculosis* peptides to bind to the murine non-classical MHC class I molecule H-2M3.

The following peptides were synthesized by standard t-BOC methodology on an Applied Biosystems Model 430 peptide synthesizer: *Mycobacterium tuberculosis* (Mtb) Peptide A (SEQ ID NO:17; referred to as Peptide A or ^{F}Met-Peptide A), Peptide B (SEQ ID NO:2; referred to as Peptide B or ^{F}Met-Peptide B), Peptide C (SEQ ID NO:4; referred to as Peptide C or ^{F}Met-Peptide C), Peptide D (SEQ ID NO:14; referred to as Peptide D or ^{F}Met-Peptide D), Peptide E (SEQ ID NO:6; referred to as Peptide E or ^{F}Met-Peptide E); Peptide F (SEQ ID NO:18; referred to as Peptide F or ^{F}Met-Peptide F); Peptide G (SEQ ID NO:19; referred to as Peptide G or ^{F}Met-Peptide G); Peptide H (SEQ ID NO:20; referred to as Peptide H or ^{F}Met-Peptide H); murine mitochondrial ND-1 peptide (SEQ ID NO:21; referred to as Peptide ND-1 or ^{F}Met-Peptide ND-1); and Bacillus cereus β lactamase peptide (SEQ ID NO:22; BlaZ

or ^{F}Met-BlaZ). Each peptide was synthesized with either an amino terminal formylated methionine (^{F}Met-Peptide) or an amino terminal non-formylated methionine. The peptides were purified by high pressure liquid chromatography and the sequence confirmed by mass spectrometry. After lyophilization the peptides were reconstituted in DMSO to a concentration of 1mM.

The ability of these peptides to bind to the peptide binding region of the murine non-classical MHC molecule, H-2M3, was determined by the method of Vyas et al. (*J. Expt. Med.* 179:155-165, 1994 and *J. Immunol.* 149:3605-3611, 1992). The theoretical basis of this assay is that ^{F}Met-peptides capable of binding to H-2M3 will stabilize expression of this molecule on the surface of cells cultured overnight at 27°C. An increase in stability of the exogenously added peptide complexed to H-2M3 can be detected using the antibody 28-14-8, which binds to a region distinct from peptide binding residues, in the H-2M3/L^{d} chimeric molecule. The cell line used was derived by Vyas et al. (*ibid.*) and comprises a fibroblast line from BIO.CAS2 mice transfected with a gene encoding a chimeric H-2M3/L^{d} gene. The cells were cultured at 37°C with 100 units/ml of γ-interferon for 24 hours and then ^{F}Met-Peptide A, ^{F}Met-Peptide B, ^{F}Met-Peptide C, ^{F}Met-Peptide D, ^{F}Met-Peptide E, ^{F}Met-Peptide F or ^{F}Met-BlaZ, were added to a final concentration of 1-20μM to parallel cultures that were incubated for an additional 16-24 hours at 27°C. Stabilization of the H-2M3/L^{d} chimeric molecule was

determined by immunofluorescence staining and flow microfluorometry. In brief, cells were harvested following trypsinization and, after extensive washing, 10^6 cells in 100μl of phosphate buffered saline (PBS) was incubated on ice for 30 minutes with an equal volume of diluted 28-14-8 monoclonal antibody. The cells were then washed twice with PBS and then incubated for another 30 min. on ice with goat anti-mouse immunoglobulin conjugated with fluorescein isothiocyanate. Flow cytometry was performed on an EPICS profile (Coulter Electronics) using standard methodology.

Fig. 1 illustrates the fluorescence profiles obtained for those cells that were incubated with DMSO alone (negative control); ^{F}Met-BlaZ (positive control); and ^{F}Met-Peptide A through ^{F}Met-Peptide F. In each plot, profiles for cells stained without (open curve) or with (filled curve) the 28-14-8 antibody are presented. A shift to the right in the filled curve is indicative of increased H-2M3/L^d expression as a consequence of the exogenously added peptide binding to this molecule. The results indicate that ^{F}Met-Peptide B, ^{F}Met-Peptide C and ^{F}Met-Peptide E, but not ^{F}Met-Peptide A or ^{F}Met-Peptide F, caused a significant increase in the level of H-2M3/L^d expression.

The mean linear fluorescence signals obtained for cells incubated with ^{F}Met forms of Peptides A through Peptide H and ND-1 is plotted in the bar graph illustrated in Fig. 2. A comparison of the mean linear fluorescence signal obtained for cells incubated with the positive control ^{F}Met-Peptide ND-1 and those obtained for cells

incubated with the various Mtb peptides indicates that ^{F}Met Peptides B, C, D and E led to a significant increase in the H-2M3/L^d expression.

The mean linear fluorescence signals obtained for cells incubated with ^{F}Met forms of the Mtb Peptides B, C and E, and ^{F}Met-BlaZ were compared with the values obtained using non-formylated forms of the same peptides and are plotted together in Fig. 3. The results indicate that the binding of the peptides to H-2M3 requires the presence of an amino terminal formylated methionine residue. In summary, this data demonstrates that the ^{F}Met Peptides B, C, D and E can bind to H-2M3 and this binding is dependent on the presence of an amino terminal formylated methionine residue.

Example 2

This example demonstrates the ability of Mtb peptides to stimulate cytotoxic T cells in such a manner that the T cells are able to kill Mtb-infected macrophage cells.

A. In Vitro Restimulation of Mtb primed T cells

Numerous C57Bl6/J x BALB/c F1 mice were infected with the live Mtb strain H37Ra (Mtb-H37Ra) through intrasplenic injection. The mice were then boosted twice with Mtb-H37Ra by subcutaneous administration at monthly intervals. Spleen cell suspensions were prepared from the infected mice by NH_4Cl-lysis of erythrocytes, followed by two PBS washes. Separate samples of dendritic cell-enriched spleen cells (isolated by adherence of the spleen cells to plastic and growth in murine recombinant granulocyte macrophage colony

stimulating factor) prepared from non-immunized syngeneic mice were pulsed with either 10 μM ^{F}Met-Peptide B, ^{F}Met-Peptide C, ^{F}Met-Peptide D or ^{F}Met-Peptide E for 18 hours and then irradiated with 3000 Rads. Each sample of pulsed cells were mixed with the spleen cells isolated from the Mtb-H37Ra-immunized mice and, as a control, from non-immunized mice. Additional controls were also prepared that comprised non-pulsed syngeneic splenic dendritic cells mixed with spleen cells isolated from Mtb-H37Ra-immunized mice. The cell mixtures were cultured in upright tissue culture flasks in Dulbeccos Modified Eagles Medium (DMEM) containing 10% fetal calf serum (FCS) and interleukin-2 (IL-2) and cultured for 5 days.

Chromium release assays were performed to determine if the cells isolated from Mtb-H37Ra-infected mice, that had been mixed with the pulsed splenic dendritic cells (i.e., the effector cell population), could kill the target cells. Target cells were prepared by infecting syngeneic bone marrow macrophages with Mtb-H37Rv at a multiplicity of infection of 1:1 for 7 days. Control target cell samples were also prepared using syngeneic bone marrow macrophages not infected with Mtb-H37Rv. The target cells were then incubated with ^{51}Cr for 1 hour. A fixed number (5 X 10^{3} cells per well) of labeled target cells were then added to decreasing numbers of the mixed population of splenic dendritic cells described immediately above. After 4 hours, supernatants were harvested and the perc ntage

specific lysis calculated from the amount of ^{51}Cr in the supernatant.

The percent lysis was determined for each ratio of effector to target cells (E:T) and the resulting curve is illustrated in Fig. 4. The results indicate that samples containing cells that had been pulsed with the ^{F}Met-Peptides B, C D and E readily killed target cells infected with Mtb. In the same experiment, control samples showed no killing of Mtb-infected or uninfected target cells. Thus, each of the peptides described in this experiment were able to stimulate cytotoxic T cells capable of killing Mtb-infected cells.

B. <u>In Vitro restimulation of Peptide Primed T cells</u>

In a second experiment, separate samples of spleen cells were prepared as described in Section A from mice immunized with syngeneic splenic dendritic cells that had been pulsed overnight with ^{F}Met-Peptide B, ^{F}Met-Peptide C or ^{F}Met-Peptide E. The mice were immunized twice with about 2 to 4 X 10^5 splenic dendritic cells per mouse, first intrasplenically and then one month later intravenously. Control mice received non-pulsed syngeneic splenic dendritic cells. Two weeks later, the mice were sacrificed and spleen cell suspensions were prepared. The spleen cells were cultured for 5 days with syngeneic splenic dendritic cells that had been pulsed with either ^{F}Met-Peptide B, ^{F}Met-Peptide C, or ^{F}Met-Peptide E, as described in Section A. Spleen cells isolated from mice immunized with ^{F}Met-Peptide B were cultured with syngeneic splenic dendritic cells

pulsed with ^{F}Met-Peptide B, and the same for samples using ^{F}Met-Peptide C and ^{F}Met-Peptide E.

Chromium release assays were performed as described in Section A and resulting curves plotted from percent specific lysis values are illustrated in Figs. 5-8. The results indicate that immunization with either ^{F}Met-Peptide B, ^{F}Met-Peptide C or ^{F}Met-Peptide E elicits cells capable of killing *Mycobacterium*-infected, but not uninfected, macrophages.

Example 3

This example demonstrates that Mtb peptides can be presented by an MHC molecule shared by more than one different haplotypes.

Spleen cells were prepared from mice immunized with Mtb-H37Ra using the method described in Example 2. The cells were then mixed with cells pulsed with ^{F}Met-Peptide B, ^{F}Met-Peptide C or ^{F}Met-Peptide E, or non-pulsed splenic dendritic cells, also as described in Example 2. Chromium release assays were performed as in Example 2. The target cells, however, included Mtb-H37Rv-infected bone marrow macrophages from non-syngeneic B10.BR mice.

The resulting curves plotted from percent specific lysis values obtained from the chromium release studies are illustrated in Figs. 9-12. The results indicate that T cells from C57B16/J mice stimulated with ^{F}Met-Peptide B, ^{F}Met-Peptide C or ^{F}Met-Peptide E, killed Mtb-infected B10.BR macrophages. Thus, the MHC molecule that presents the

peptides used in this experiment is common to both C57Bl6/J and B10.BR, which differ in haplotype.

Example 4

This example demonstrates that Mtb peptides containing amino terminal formylated methionine residues are protective against Mtb infection.

The results disclosed in Examples 1-3 indicated that Mtb peptides could elicit cytotoxic reactivity against Mtb-infected cells. As such, the ability of the same peptides to elicit protective immunity was examined in a mouse model of Mtb infection.

Eight groups of C57Bl6/J mice (5 mice per group) were prepared: Group 1 comprised a control group which did not receive any intrasplenic injection of syngeneic splenic dendritic cells; Group 2 comprised a control group in which the mice were injected with syngeneic splenic dendritic cells that were not pulsed with peptide; Group 3 comprised a control group in which the mice were injected with syngeneic splenic dendritic cells that had been pulsed with the ^{F}Met-Peptide ND-1, which binds well to non-classical MHC; Group 4 comprised an experimental group in which mice were injected with syngeneic splenic dendritic cells that had been pulsed with ^{F}Met-Peptide A, that according to the results presented in Example 1, does not bind to H-2M3; Group 5 comprised an experimental group in which mice were injected with syngeneic splenic dendritic cells that had been pulsed with ^{F}Met-Peptide B; Group 6 comprised an experimental group in which mice were injected with

syngeneic splenic dendritic cells that had been pulsed with ^{F}Met-Peptide C; Group 7 comprised an experimental group in which mice were injected with syngeneic splenic dendritic cells that had been pulsed with ^{F}Met-Peptide D; Group 8 comprised an experimental group in which mice were injected with syngeneic splenic dendritic cells that had been pulsed with ^{F}Met-Peptide E.

Ten days after peptide immunization, each mouse was given an aerosol challenge of virulent Mtb-H37Rv strain using a Middlebrook Airborne Infection Apparatus (Middlebrook Co, Glasgow, Scotland) at a dose designed to deliver 50 to 100 viable bacteria into the airways of each mouse. Thirty days after challenge, the mice were sacrificed and the number of viable Mtb bacteria was determined. Bacteria counts were obtained using the following method. The spleen and lungs were removed from each mouse and homogenized. Each homogenate was serially diluted (10-fold dilutions) in sterile PBS and plated on nutrient 7H11 Middlebrook agar quadrant plates. The plates were maintained at 37°C in humidified incubators for 3 to 4 weeks. The number of bacterial colonies present on each plate was then counted to determine the number of bacteria present in each organ at the time of sacrifice. These values indicated the effectiveness of the immunizing peptide on the immune response to Mtb bacteria.

The results are presented as the mean number of bacteria per spleen or lung for each group and are shown in Figs. 13 and 14, respectively. The results indicate that

mice immunized with either ^{F}Met-Peptide B, ^{F}Met-Peptide C or ^{F}Met-Peptide E had significantly lower Mtb bacterial counts in their spleens, compared to control mice or those immunized with ^{F}Met-Peptide A, ^{F}Met-Peptide D or ^{F}Met-Peptide ND-1. Mice immunized with ^{F}Met-Peptide B or ^{F}Met-Peptide E also had significantly fewer Mtb bacteria counts in their lungs, compared to controls or mice immunized with ^{F}Met-Peptide A, ^{F}Met-Peptide C, ^{F}Met-Peptide D or ^{F}Met-Peptide ND-1. Thus, immunization of mice with ^{F}Met-Peptide B, ^{F}Met-Peptide C and ^{F}Met-Peptide E elicited a protective response against the growth of Mtb in the lungs and/or spleens of mice.

Additional evidence for the protective nature of ^{F}Met-Peptide B was demonstrated by the presence of well-developed granulomas in the lungs of mice immunized with ^{F}Met-Peptide B, as shown by histological analysis. Organs were fixed in 10% neutral buffered formalin and then imbedded in paraffin. Two micron sections were cut and stained with hematoxylin and eosin. Control groups failed to show the presence of such granulomas.

Taken together, the results from the foregoing experiments described in Examples 1-4 indicate that: (1) ^{F}Met-Peptide B, ^{F}Met-Peptide C, ^{F}Met-Peptide D and ^{F}Met-Peptide E bind to MHC class I molecules, thereby stimulating T cells capable of killing Mtb-infected cells, thereby indicating that the synthetic peptides used in these experiments are indistinguishable, by T cells, from the naturally-occurring Mtb peptides which bind to MHC

class I molecules; and (2) that these ^{F}Met-Peptides generate a protective response against the growth of Mtb in mice.

Example 5

This example demonstrates that human peripheral blood T cells stimulated with Mtb peptides can lyse Mtb-infected macrophages.

Blood was collected from a human patient with active pulmonary tuberculosis and peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density centrifugation using standard methods. Monocytes were isolated from the population of purified PBMCs by adherence to plastic culture flasks. The monocytes were then cultured in DMEM with 10% normal human blood group AB serum plus 100 U/ml of human recombinant macrophage colony stimulating factor (M-CSF). Two separate samples were prepared using the purified monocytes. A first sample of monocytes was grown in culture for 9 days and then infected with Mtb-H37Ra and used as target cells 5 days after infection in chromium release assays as described in Example 2. Control samples of uninfected monocytes were also prepared.

A second sample of monocytes was pulsed overnight with 10 μM ^{F}Met-Peptide B or ^{F}Met-Peptide E and irradiated with 2500 Rads. The pulsed monocytes were then added to the monocyte-depleted population of PBMCs, at a 2:1 ratio of cells from the population of depleted PBMCs to pulsed monocytes, to create a mixed responder/stimulator population. This mixed responder/stimulator population was

then restimulated by adding peptide pulsed monocytes 7 days later.

Chromium release assays were performed (as described in Example 2) to determine the cytotoxic activity of the mixed population of responder/stimulator cells for the Mtb-infected monocytes. The percent specific lysis values obtained for each sample are plotted in curves illustrated in Figs. 15 and 16. The results indicate that *in vitro* stimulation with ^{F}Met-Peptide B and ^{F}Met-Peptide E generated T cells that are capable of killing human Mtb-infected monocytes. The level of cytotoxic T cell activity generated by ^{F}Met-Peptide B and ^{F}Met-Peptide E was similar. Thus, ^{F}Met-Peptide B and ^{F}Met-Peptide E, which showed protective activity in mice, are able to elicit cytotoxic reactivity to Mtb-infected cells, in PBMCs from a human patient infected with Mtb.

SEQUENCE LISTING

The following Sequence Listing is submitted pursuant to 37 CFR §1.821. A copy in computer readable form is also submitted herewith.

Applicants assert pursuant to 37 CFR §1.821(f) that the content of the paper and computer readable copies of SEQ ID NO:1 through SEQ ID NO:22 submitted herewith are the same.

(1) GENERAL INFORMATION:

(i) APPLICANT: Potter, Terence A.
Dow, Steve W.
Orme, Ian R.

(ii) TITLE OF INVENTION: ISOLATED FORMYLATED BACTERIAL PEPTIDES, NUCLEIC ACID MOLECULES AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 80203

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kovarik, Joseph E.
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(C) REFERENCE/DOCKET NUMBER: 2879-30-PCT

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATG GCC AAT CCG TTC GTT AAA GCC TGG AAG TAC
33
Met Ala Asn Pro Phe Val Lys Ala Trp Lys Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Asn Pro Phe Val Lys Ala Trp Lys Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG CAG CTT GTT GAC AGG GTT CGT GGC GCC GTC
33
Met Gln Leu Val Asp Arg Val Arg Gly Ala Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gln Leu Val Asp Arg Val Arg Gly Ala Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG ACG TTC TTC GAA CAG GTG CGA AGG TTG CGG
33
Met Thr Phe Phe Glu Gln Val Arg Arg Leu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Thr Phe Phe Glu Gln Val Arg Arg Leu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATG GCC AAT CCG TTC GTT AAA GCC
24
Met Ala Asn Pro Phe Val Lys Ala
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Asn Pro Phe Val Lys Ala
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG CAG CTT GTT GAC AGG GTT CGT
24
Met Gln Leu Val Asp Arg Val Arg
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Gln Leu Val Asp Arg Val Arg
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATG ACG TTC TTC GAA CAG GTG CGA
24
Met Thr Phe Phe Glu Gln Val Arg
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Thr Phe Phe Glu Gln Val Arg
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATG GCC ACC ACC CTT CCC GTT CAG CGC CAC CCG
33
Met Ala Thr Thr Leu Pro Val Gln Arg His Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ala Thr Thr Leu Pro Val Gln Arg His Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATG GCC ACC ACC CTT CCC GTT CAG
24
Met Ala Thr Thr Leu Pro Val Gln
1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ala Thr Thr Leu Pro Val Gln
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Ala Lys Val Asn Ile Lys Pro Leu Gln Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Lys Arg Gly Leu Thr Val Ala Val Ala Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Thr Asp Val Ser Arg Lys Ile
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Lys Ile Arg Leu His Thr Leu
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Phe Phe Ile Asn Ile Leu Thr
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Phe Val Leu Asn Lys Phe Phe
1 5

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to thos skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims:

What is claimed is:

1. An isolated antigenic peptide having an amino-terminal formylated methionine, said peptide being capable of protecting an animal against *Mycobacterium* infection.

2. The peptide of Claim 1, wherein said *Mycobacterium* is a species selected from the group consisting of *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium avium* and *Mycobacterium bovis*.

3. The peptide of Claim 1, wherein said peptide is capable of binding to an MHC molecule in such a manner that said peptide bound to said MHC molecule creates an epitope recognized by a T cell receptor.

4. The peptide of Claim 1, wherein said peptide is capable of binding to an MHC class I molecule in such a manner that said peptide bound to said MHC molecule creates an epitope recognized by a T cell receptor, said MHC molecule being capable of binding to a naturally-occurring *Mycobacterium* peptide.

5. The peptide of Claim 1, wherein said peptide comprises a length of from about 5 amino acids to about 20 amino acids.

6. The peptide of Claim 1, wherein said peptide comprises a length of from about 6 amino acids to about 15 amino acids.

7. The peptide of Claim 1, wherein said peptide comprises a length of from about 8 amino acids to about 11 amino acids.

8. The peptide of Claim 1, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 10 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

9. The peptide of Claim 1, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 25 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

10. The peptide of Claim 1, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 50 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

11. The peptide of Claim 1, wherein said peptide is derived from a protein selected from the group consisting of an animo acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

12. The peptide of Claim 1, wherein said peptide is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene selected from the group consisting of a nucleic acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M.*

Tuberculosis GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

13. The peptide of Claim 12, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

14. An isolated *Mycobacterium* peptide having an amino-terminal formylated methionine.

15. The peptide of Claim 14, wherein said peptide is capable of protecting an animal from infection by *Mycobacterium*.

16. The peptide of Claim 14, wherein said *Mycobacterium* is selected from the group consisting of *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium avium* and *Mycobacterium bovis*.

17. The peptide of Claim 14, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 10 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

18. The peptide of Claim 14, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 25 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

19. The peptide of Claim 14, wherein said peptide, when administered to an animal, is capable of limiting the growth of *Mycobacterium* bacteria by at least about 50 percent compared to the growth of *Mycobacterium* bacteria in animals not receiving said peptide.

20. The peptide of Claim 14, wherein said peptide comprises a length of from about 5 amino acids to about 20 amino acids.

21. The peptide of Claim 14, wherein said peptide comprises a length of from about 7 amino acids to about 15 amino acids.

22. The peptide of Claim 14, wherein said peptide comprises a length of from about 8 amino acids to about 11 amino acids.

23. The peptide of Claim 14, wherein said peptide is derived from a protein selected from the group consisting of an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

24. The peptide of Claim 14, wherein said peptide is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene selected from the group consisting of a nucleic acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M.*

Tuberculosis GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

25. The peptide of Claim 24, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

26. An isolated nucleic acid molecule having a sequence encoding a peptide having an amino-terminal formylated methionine when said nucleic acid molecule is expressed in a bacteria cell, said peptide being capable of protecting an animal from *Mycobacterium* infection.

27. The nucleic acid molecule of Claim 26, wherein said nucleic acid molecule encodes a *Mycobacterium* peptide.

28. The nucleic acid molecule of Claim 26, wherein said nucleic acid molecule encodes a peptide derived from a protein selected from the group consisting of an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

29. The nucleic acid molecule of Claim 26, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

30. The nucleic acid molecule of Claim 26, wherein said sequence is operatively linked to one or more transcription control sequences capable of controlling transcription in a bacteria to form a recombinant molecule.

31. The nucleic acid molecule of Claim 26, wherein said sequence is operatively linked to one or more transcription control sequences that control the transcription of a protein selected from the group consisting of listeriolysin, internalin, and *Mycobacterium* heat shock protein.

32. The nucleic acid molecule of Claim 30, wherein said recombinant molecule is transformed into an attenuated pathogenic bacteria.

33. The nucleic acid molecule of Claim 32, wherein said recombinant molecule is transformed into an attenuated bacteria having a genus selected from the group consisting of *Mycobacterium*, *Listeria*, *E. coli*, *Bacillus*, *Pseudomonas* and *Salmonella*.

34. The nucleic acid molecule of Claim 32, wherein said recombinant molecule is transformed into the *Mycobacterium* BCG.

35. An isolated nucleic acid molecule having a sequence encoding a *Mycobacterium* peptide having an amino-terminal formylated methionine when said nucleic acid molecule is expressed in a bacterial cell.

36. The nucleic acid molecule of Claim 35, wherein said nucleic acid molecule encodes a peptid derived from a protein selected from the group consisting of an amino acid

sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

37. The nucleic acid molecule of Claim 35, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions with a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

38. The nucleic acid molecule of Claim 35, wherein said sequence is operatively linked to one or more transcription control sequences capable of controlling transcription in a bacteria to form a recombinant molecule.

39. The nucleic acid molecule of Claim 35, wherein said sequence is operatively linked to one or more transcription control sequences that control the transcription of a protein selected from the group consisting of listeriolysin, internalin, and *Mycobacterium* heat shock protein.

40. The nucleic acid molecule of Claim 38, wherein said recombinant molecule is transformed into an attenuated pathogenic bacteria.

41. The nucleic acid molecule of Claim 40, wherein said recombinant molecule is transformed into an attenuated bacteria selected from the group consisting of *Mycobacterium*, *Listeria*, *E. coli*, *Bacillus*, *Pseudomonas* and *Salmonella*.

42. The nucleic acid molecule of Claim 38, wherein said recombinant molecule is transformed into the *Mycobacterium* BCG.

43. A recombinant cell vaccine comprising a bacteria cell containing a recombinant molecule comprising a nucleic acid molecule encoding an antigenic peptide capable of protecting an animal from *Mycobacterium* infection, said nucleic acid molecule being operatively linked to one or more transcription control sequences.

44. The recombinant cell vaccine of Claim 43, wherein said bacteria cell is an attenuated bacteria cell.

45. The recombinant cell vaccine of Claim 43, wherein said bacteria cell is selected from the group consisting of an attenuated *Mycobacterium*, *Listeria*, *E. coli*, *Bacillus*, *Pseudomonas* and *Salmonella*.

46. The recombinant cell vaccine of Claim 43, wherein said bacteria cell is the *Mycobacterium*, BCG.

47. The recombinant cell vaccine of Claim 43, wherein said peptide comprises an amino-terminal formylated methionine.

48. The recombinant cell vaccine of Claim 43, wherein said peptide is derived from a *Mycobacterium* protein.

49. The recombinant cell vaccine of Claim 43, wherein said nucleic acid molecule encodes a peptide derived from a protein selected from the group consisting of an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016

X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

50. The recombinant cell vaccine of Claim 43, wherein said nucleic acid molecule hybridizes under stringent hybridization conditions to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

51. The recombinant cell vaccine of Claim 43, wherein said nucleic acid molecule is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

52. The recombinant cell vaccine of Claim 43, wherein said transcription control sequence is a transcription control sequence that controls the transcription of a protein selected from the group consisting of listeriolysin, internalin, and *Mycobacterium* heat shock protein.

53. The recombinant cell vaccine of Claim 43, wherein said recombinant cell vaccine further comprises a nucleic acid molecule encoding an adjuvant.

54. The recombinant cell vaccine of Claim 53, wherein said adjuvant comprises a nucleic acid molecule encoding a cytokine.

55. A therapeutic composition to protect an animal from infection by an intracellular pathogen, said composition comprising: (1) an isolated antigenic peptide having an amino-terminal formylated methionine; and (2) a

pharmaceutically acceptable carrier, wherein said antigenic peptid is capable of protecting an animal from *Mycobacterium* infection.

56. The therapeutic composition of Claim 55, wherein said composition is administered to protect an animal from *Mycobacterium* infection.

57. The therapeutic composition of Claim 55, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution and a delivery vehicle

58. The therapeutic composition of Claim 57, wherein said delivery vehicle is selected from the group consisting of polymeric controlled release formulations, bolus preparations, artificial and natural lipid-containing compositions, and transdermal delivery systems, oils, esters, and glycols.

59. The therapeutic composition of Claim 57, wherein said delivery vehicle comprises a liposome.

60. The therapeutic composition of Claim 57, wherein said delivery vehicle is a liposome comprising a ligand capable of targeting said liposomes to an antigen presenting cell.

61. The therapeutic composition of Claim 55, wherein said composition further comprises an adjuvant.

62. The therapeutic composition of Claim 55, wherein said composition further comprises an adjuvant selected from the group consisting of aluminum-bas d salts, calcium-based salts, silica and gamma interferon.

63. The therapeutic composition of Claim 55, wher in said peptide is deriv d from a *Mycobacterium* protein.

64. The therapeutic composition of Claim 55, wherein said peptide is derived from a protein selected from the group consisting of an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

65. The therapeutic composition of Claim 55, wherein peptide is encoded by a nucleic acid molecule that hybridizes to a nucleic acid molecule selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

66. An isolated antigenic peptide selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and SEQ ID NO:16, wherein said peptide has an amino-terminal formylated methionine.

67. A formula comprising an isolated antigenic peptide selected from a group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, and combinations thereof.

68. A method to protect an animal from an intracellular pathogen, comprising administering to an animal a therapeutic composition comprising an isolated antigenic peptide having an amino-terminal formylated

methionine, said peptide being capable of protecting an animal from *Mycobacterium* infection.

69. The method of Claim 68, wherein said step of administering comprises delivering said therapeutic composition by a parenteral route selected from the group consisting of subcutaneous, intradermal, aerosol and intramuscular routes.

70. The method of Claim 68, wherein said therapeutic composition further comprises a pharmaceutically acceptable carrier.

71. The method of Claim 70, wherein said carrier is selected from the group consisting of an aqueous physiologically balanced solution and a delivery vehicle.

72. The method of Claim 71, wherein said delivery vehicle is selected from the group consisting of biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, liposomes, lipospheres, and transdermal delivery systems.

73. The method of Claim 68, wherein said intracellular pathogen is *Mycobacterium*.

74. The method of Claim 68, wherein said peptide is derived from a protein selected from the group consisting of an amino acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

75. The method of Claim 68, wherein said peptide is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene selected from the group consisting of a nucleic acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

76. The method of Claim 75, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

77. The method of Claim 68, wherein said peptide comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:16, and combinations thereof.

78. A method to protect an animal from an intracellular pathogen, comprising administering to an animal a therapeutic composition comprising a nucleic acid sequence encoding an antigenic peptide capable of protecting an animal from *Mycobacterium* infection when said nucleic acid sequence is transformed into a bacterial cell, said nucleic acid sequence being operatively linked to one or more transcription control sequences.

79. The method of Claim 78, wherein said bacterial cell is an attenuated bacteria cell.

80. The method of Claim 78, wherein said bacterial cell is selected from the group consisting of an attenuated *Mycobacterium*, *Listeria*, *E. coli*, *Bacillus*, *Pseudomonas* and *Salmonella* cell.

81. The method of Claim 78, wherein said intracellular pathogen is a *Mycobacterium*.

82. The method of Claim 78, wherein said peptide is derived from a protein selected from the group consisting of an amino acid sequence represented by M. Tuberculosis GenBank Accession No. M69187, M. Tuberculosis GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

83. The method of Claim 78, wherein said peptide is encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions to a gene selected from the group consisting of a nucleic acid sequence represented by *M. Tuberculosis* GenBank Accession No. M69187, *M. Tuberculosis* GenBank Accession No. M27016 X53898, *M. Tuberculosis* GenBank Accession No. X57229 and *M. Tuberculosis* GenBank Accession No. M76712.

84. The method of Claim 83, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:13, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 and SEQ ID NO:15.

85. The method of Claim 78, wherein said peptide comprises an amino acid sequence selected from a group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:14, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 and/or SEQ ID NO:16, and combinations thereof.

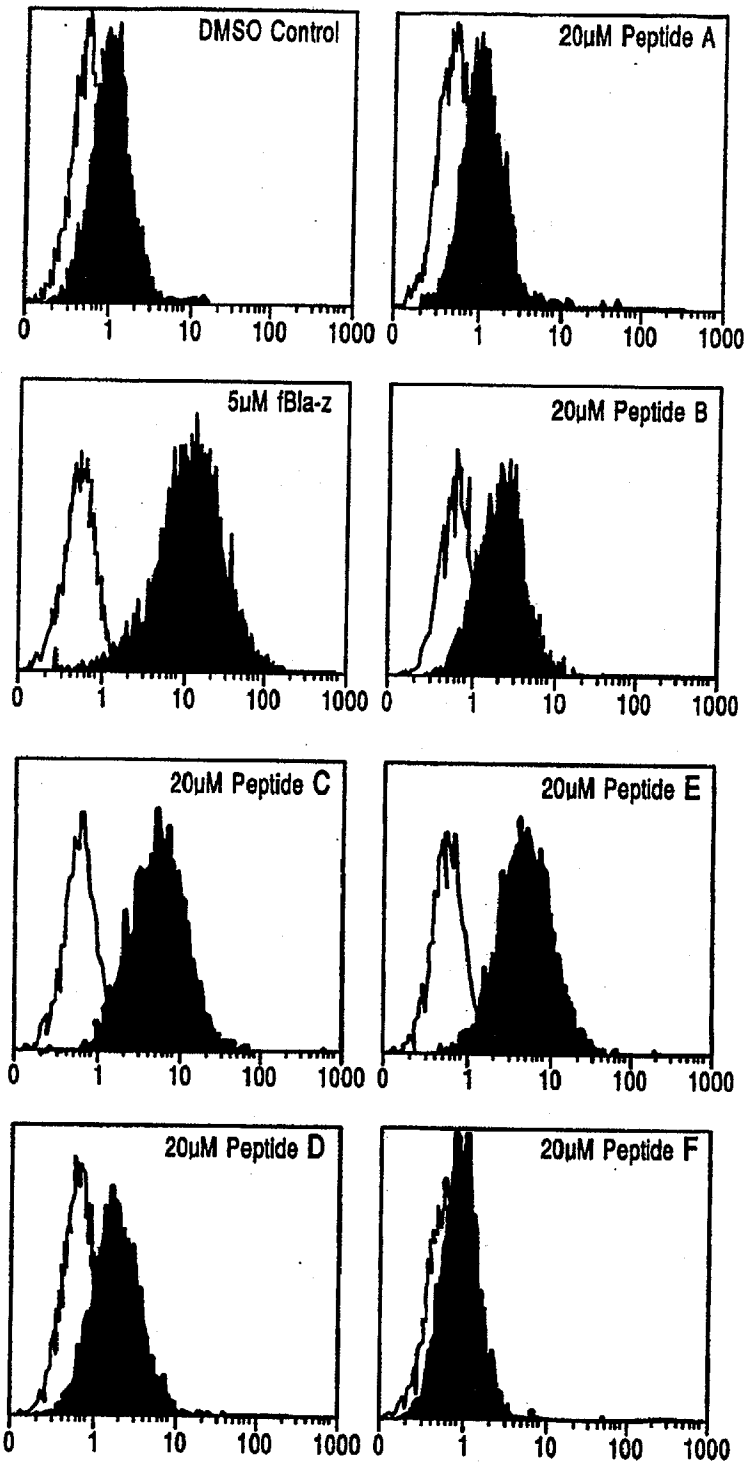


Fig. 1

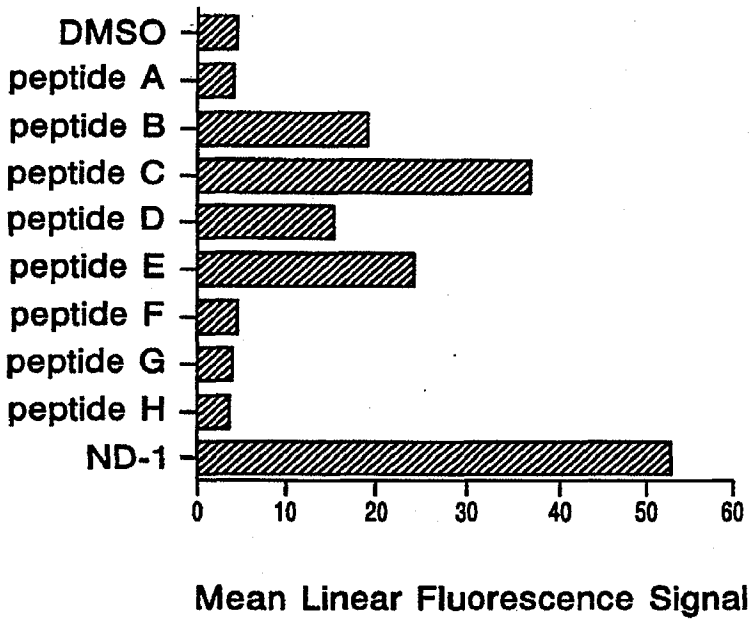


Fig. 2

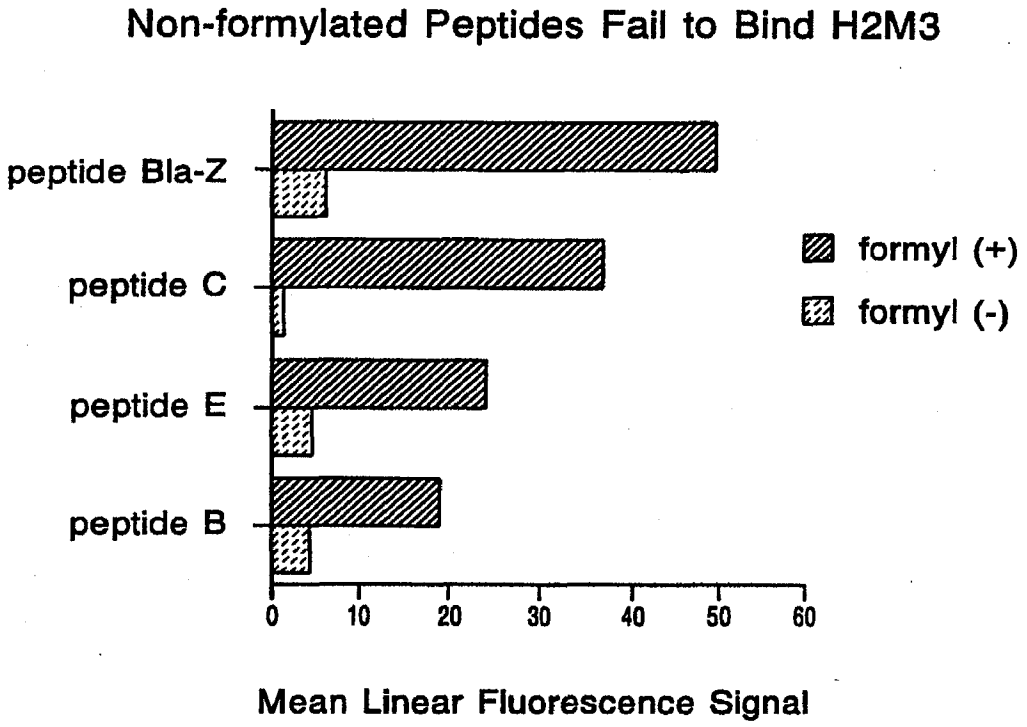


Fig. 3

Peptide Elicited CTL Activity Against H37Rv-Infected BMMØ

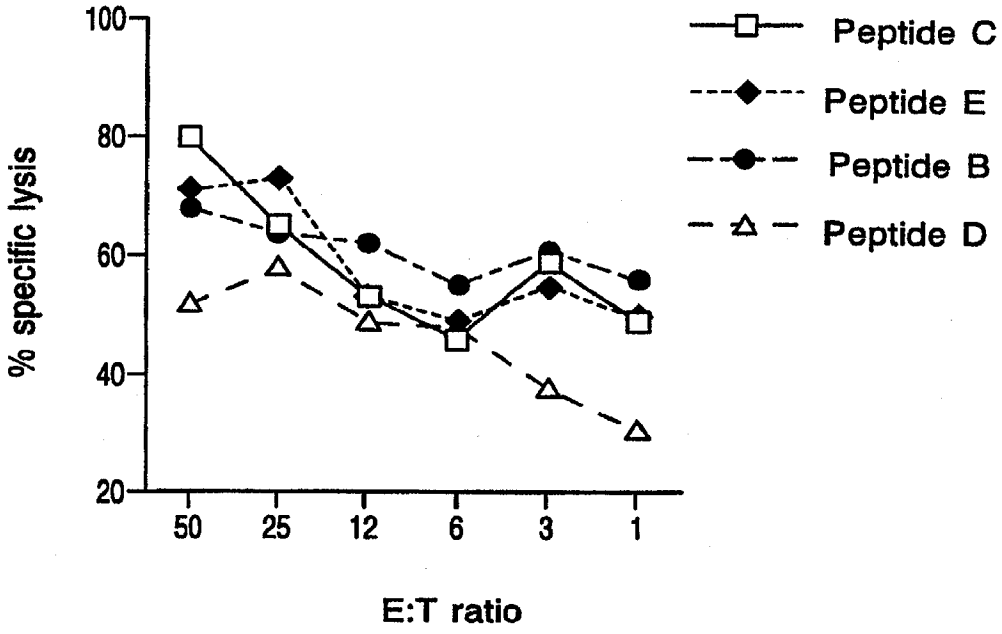


Fig. 4

CTL Elicited Without Peptide (C57B6 targets)

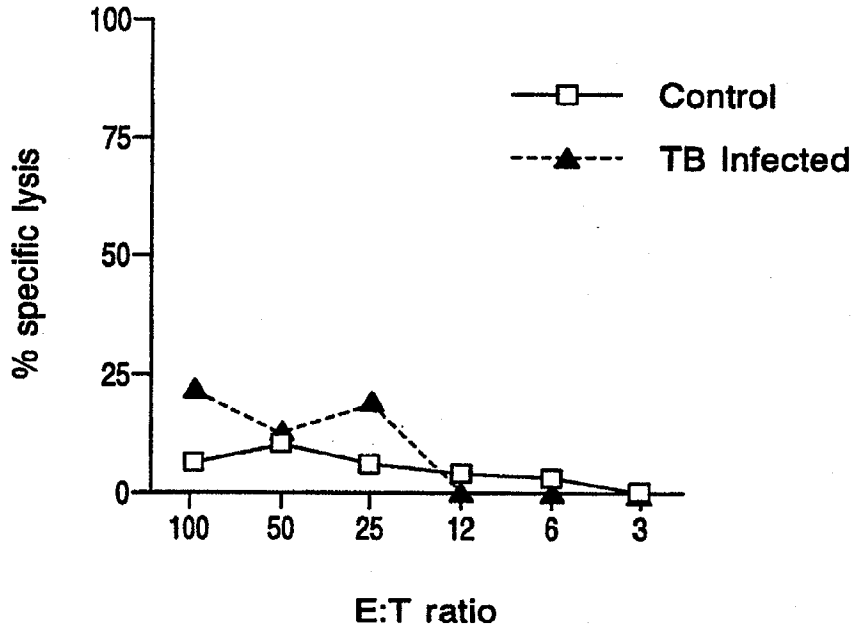


Fig. 5

Peptide B Elicited CTL (C57B6 targets)

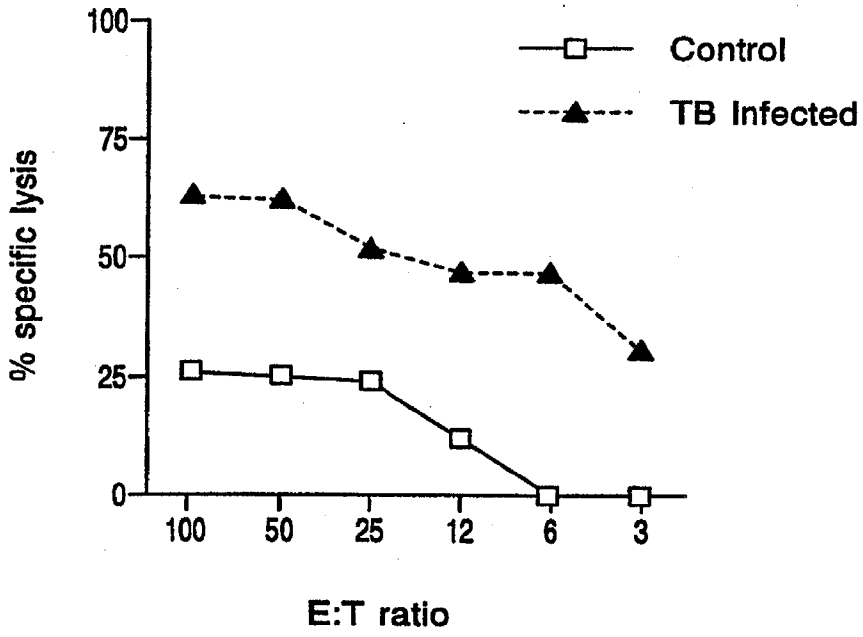


Fig. 6

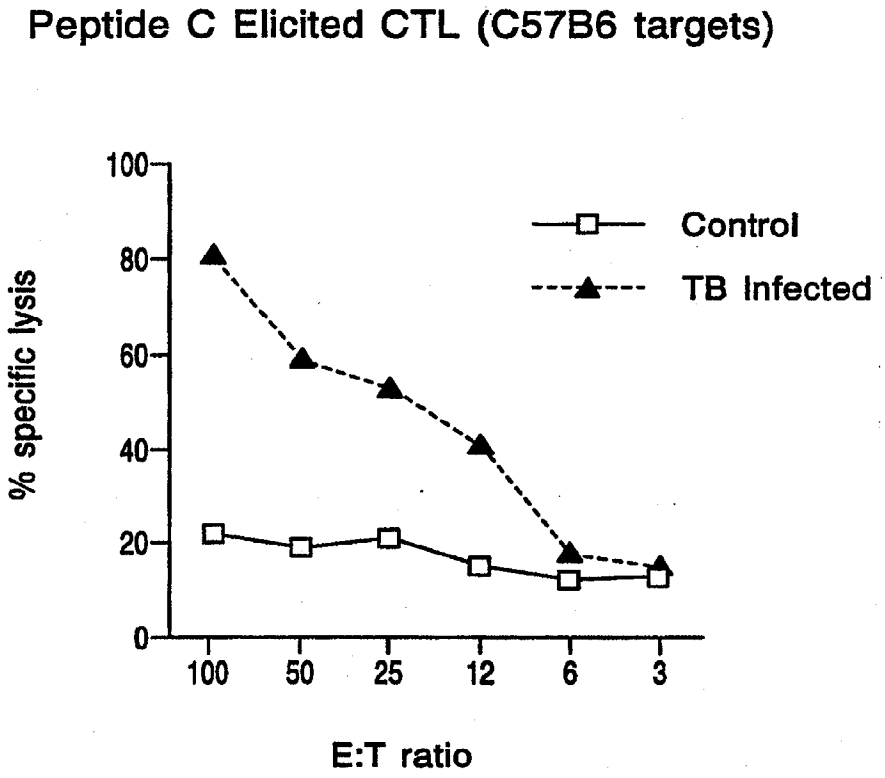


Fig. 7

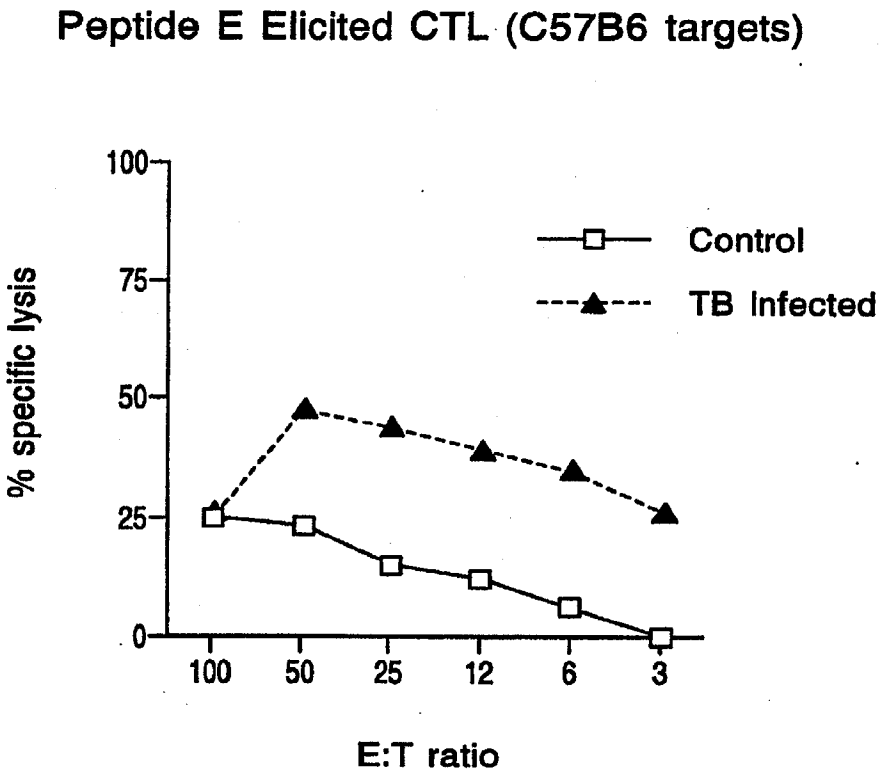


Fig. 8

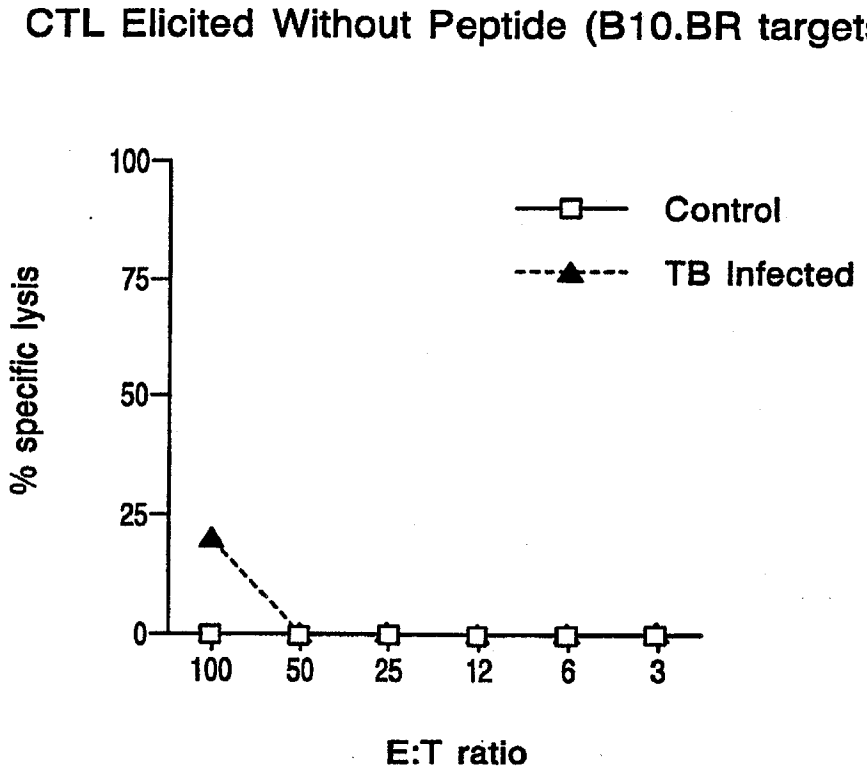


Fig. 9

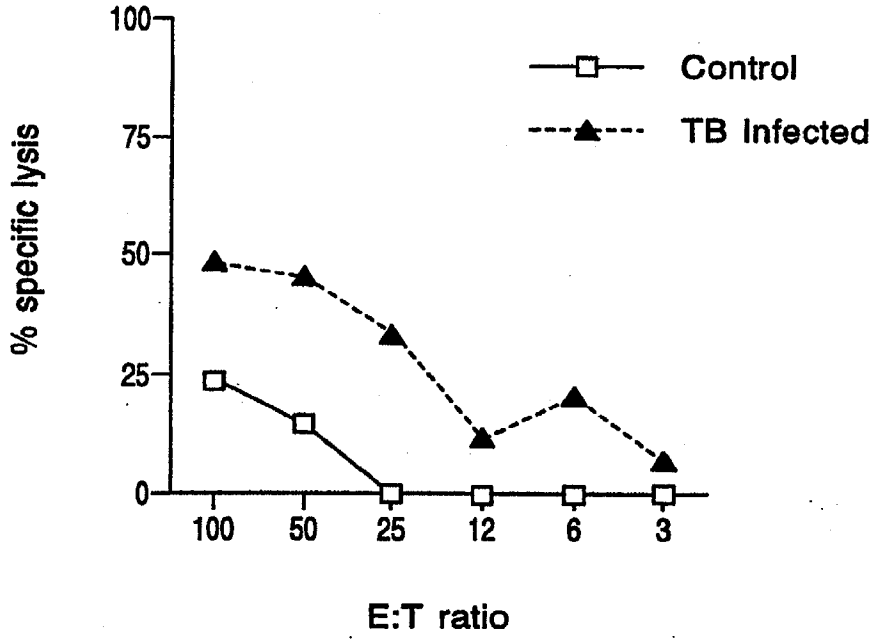


Fig. 10

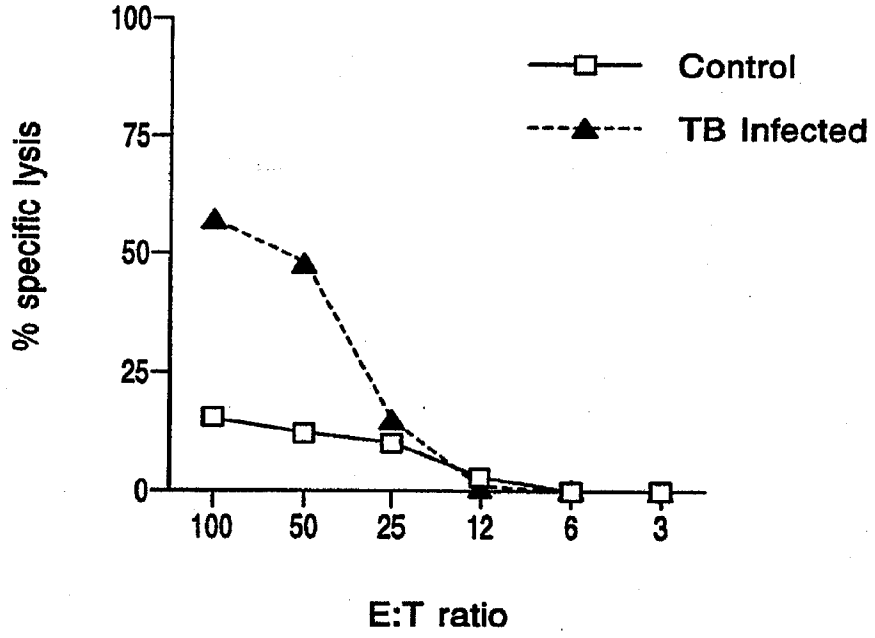


Fig. 11

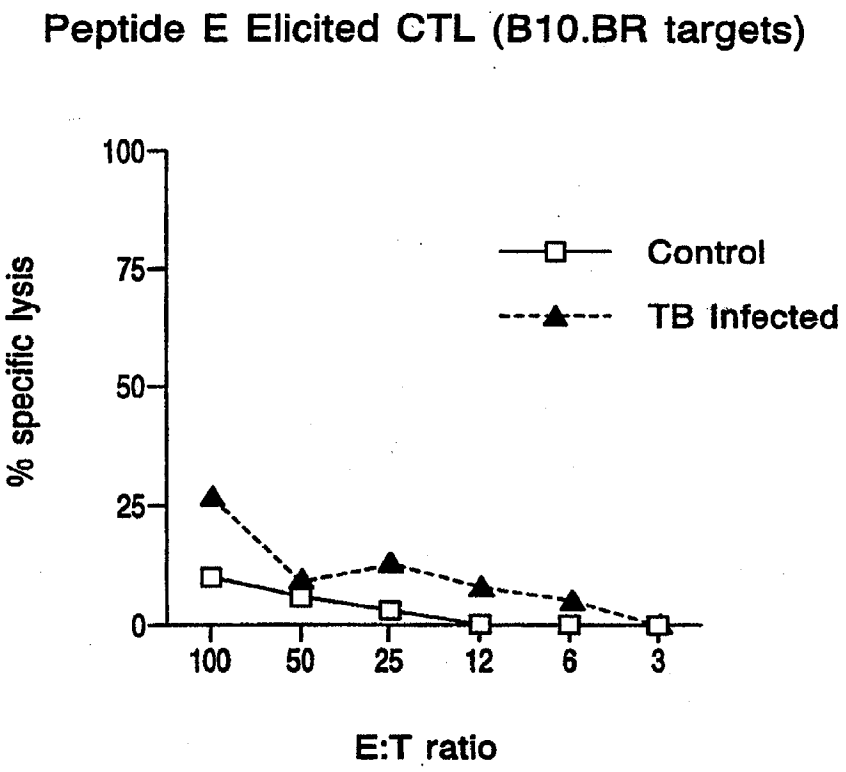
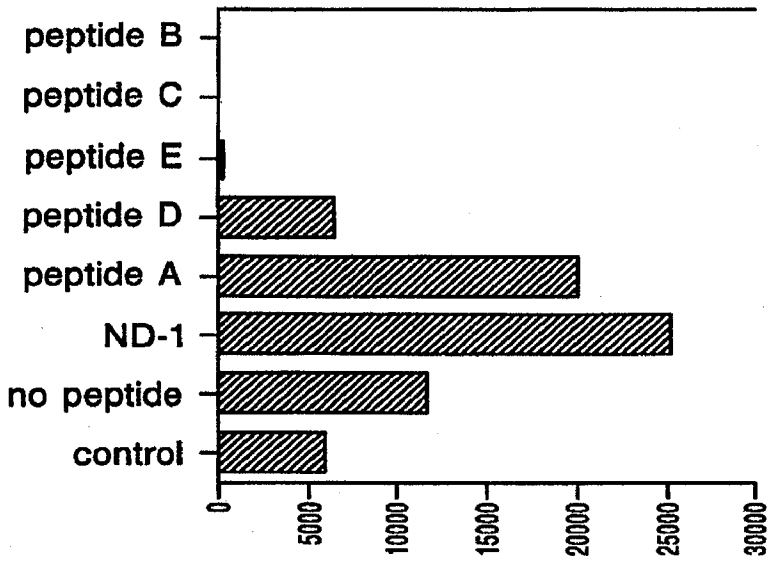


Fig. 12

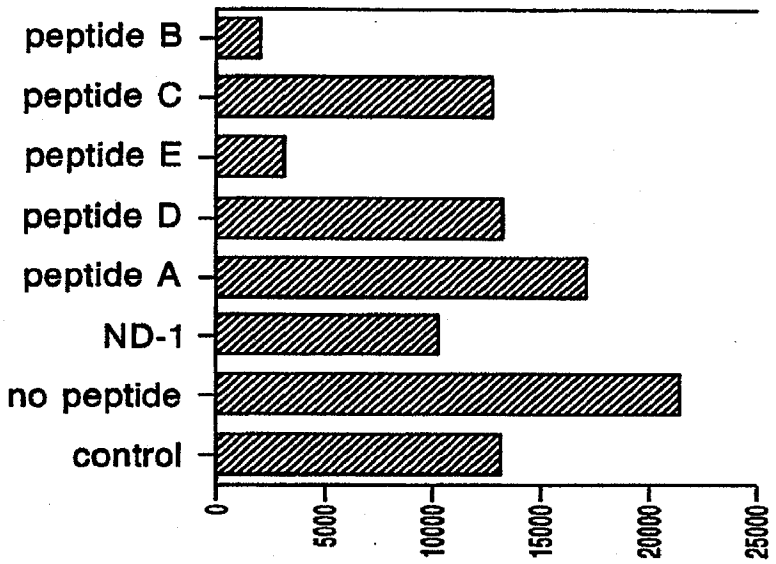
Effect of Peptide Immunization on TB Dissemination to Spleen



(viable organisms per spleen)

Fig. 13

Effect of Peptide Immunization on TB Counts in Lung



(viable organisms per lung)

Fig. 14

Peptide E Elicited CTL Activity in Human PBMC

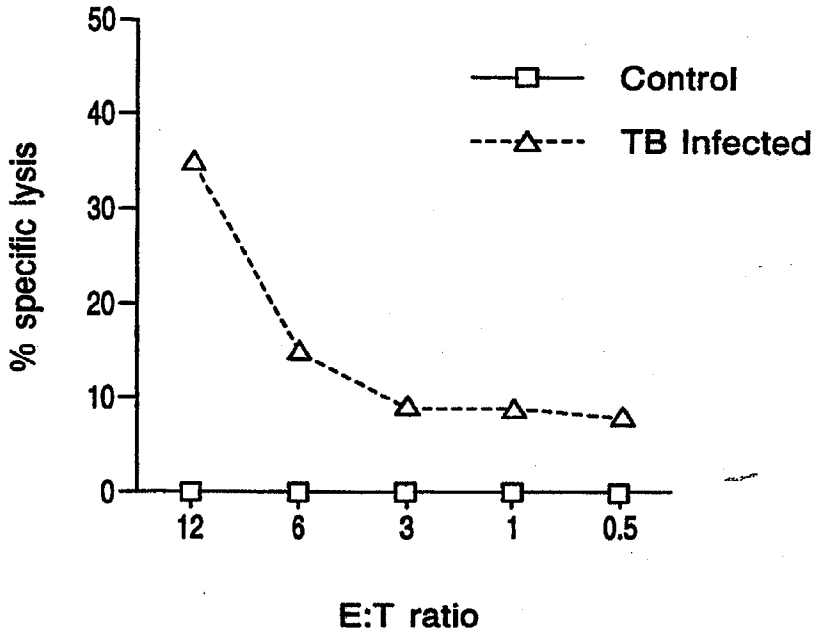


Fig. 15

Peptide B Elicited CTL Activity in Human PBMC

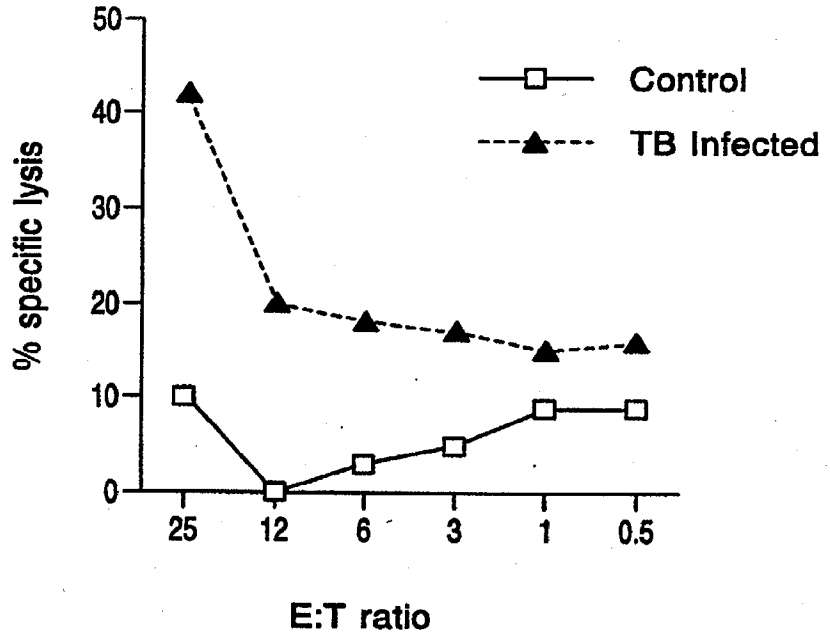


Fig. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09473

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 39/02, 39/ 04; C07K 1/00; C07H 21/02, 21/04
US CL :424/200.1, 248.1; 530/350; 536/23.1, 23.7
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/200.1, 248.1; 530/350; 536/23.1, 23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GENBANK, APS, DIALOG, MEDLINE, EMBL, CAPLUS, EMBASE, LIFESCI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y ------ A	Research Microbiology, Volume 141, issued 1990, S.P. O'Connor et al, "Nucleotide sequence of the gene encoding the 35-kDa protein of Mycobacterium tuberculosis", pages 407-423, entire document.	1-10, 13, 14-22, 25 ---------- 55-67, 11, 12, 23, 24 ---------- 68-77
Y	Nucleic Acids Research, Volume 18, No. 13, issued 1990, L. De Wit et al, "Nucleotide sequence of the 32 KDa-protein gene (antigen 85A) of Mycobacterium bovis BCG", page 3995.	1-25, 55-77

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 AUGUST 1996	Date of mailing of the international search report 02 OCT 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JENNIFER SHAVER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09473

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ------ Y	Infection and Immunity, Volume 57, No. 10, issued October 1989, "Cloning, sequence determination, and expression of a 32-kilodalton-protein gene of Mycobacterium tuberculosis", pages 3123-3130, entire document.	1-25 ------- 55-77
Y ------ A	Infection and Immunity, Volume 59, No. 10, issued September 1991, J. Content et al, "The genes coding for the antigen 85 complexes of Mycobacterium tuberculosis and Mycobacterium bovis BCG are members of a gene family: cloning, sequence determination, and genomic organization of the gene coding for antigen 85-c of M.tuberculosis", pages 3205-3212, entire document.	1-25, 55-67 ---------- 68-77
Y ------ A	Journal of Bacteriology, Volume 17, No. 4, issued February 1992, A. Verbon et al, "The 14,000 molecular-weight antigen of Mycobacterium tuberculosis is related to the alpha-crystallin family of low-molecular weight heat shock proteins", pages 1352-1359, entire document.	1-25 ------- 55-77
A	EP 0,571,911 A2 (BECTON, DICKINSON & COMPANY) 01 December 1993, entire document.	1-25, 55-77
Y	EP 0,499,003 A1 (N.V. INNOGENETICS S.A.) 19 August 1991, entire document.	1-25, 55-77

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09473

Box I Observati ns where certain claims were found unsearchable (Continuation of item 1 f first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-25, 55-77

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09473

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-25 and 55-77, drawn to antigenic peptides having an amino-terminal formylated methionine and therapeutic compositions comprising said peptides.
Group II, claim(s) 26-42, drawn to nucleic acid molecules which encode a Mycobacterium peptide.
Group III, claim(s) 43-54, drawn to recombinant cell vaccines.
Group IV, claim(s)78-85, drawn to methods for protecting an animal from an intracellular pathogen through the administration of nucleic acid.
The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The special technical feature of Group I is a peptide having an amino-terminal formylated methionine which is capable of protecting an animal against Mycobacterium infection and therapeutic compositions comprising said peptide. This peptide does not share a special technical feature with Groups II, III, IV as the peptides of Group I are different. chemically, structurally and biologically from the nucleic acid and recombinant cells of Groups II, III and IV.
The special technical feature of Group II is nucleic acid molecules which encode a Mycobacterium peptide. The nucleic acid molecules of Group II do not share a special technical feature with the recombinant cell vaccines of Group III as the nucleic acid molecules of Group II have other uses than as immunogens in a recombinant cell vaccine, i.e., the nucleic acid molecules of Group II may be utilized to produce proteins or may be used in detection methods. Further, the recombinant cell vaccines of Group III contain nucleic acid which is not included in Group II, i.e., nucleic acid which encodes an an adjuvant.
Lastly, the nucleic acid of Group IV does not share a special technical feature with that of Group II as the nucleic acid of Group II specifically encodes for a peptide having an amino-terminal formylated methionine while the therapeutic methods of Group IV do not require the use of nucleic acids which encode peptides which have an amino-terminal formylated methionine. Therefore, the method of Group IV can be performed with DNA which is not recited in Group II.
Finally, the method of Group IV utilizes nucleic acid compositions which need not be in the form of a recombinant cell as in Group III and the vaccines of Group III encode for a peptide having an amino-terminal formylated methionine which is not the case in the methods of Group IV. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.